Peroral insulin delivery : new concepts and excipients

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Chapter 1

Peroral peptide delivery and absorption challenges using novel drug delivery systems: current status

1. Introduction

Rapid developments in biotechnology have posed new challenges for pharmaceutical scientists to develop peptide and protein drugs especially for the treatment of chronic diseases at sufficient amounts and at an affordable price. Peptides and proteins are highly potent and specific in their physiological activities. However, there are severe difficulties in administration of these active agents orally. Most peptide and protein drugs are unstable in the gastrointestinal (GI) tract and have poor oral absorption due to their size and hydrophilic properties. Such low absorption can be increased by the chemical modification of the polypeptide to render it more stable and probably by increasing its lipophilicity [1]. It is well documented that peptides and proteins are easily hydrolyzed and digested by acids and enzymes in the GI tract. Hence, to deliver peptide and proteins orally, it is necessary to protect these active agents from the harsh and unfavorable GI environment.

Oral peptide drug delivery is the most desirable and the preferred method of administrating therapeutic agents. In addition, the oral medication is generally considered as the first choice for investigation

in the discovery and development of new pharmaceutical formulations due to convenience in administration, patient compliance and cost effective manufacturing process. However, not all new drugs and especially hydrophilic peptide and protein drugs can be administered orally, because of their sensitivity to gastric acid, their instability to gastrointestinal enzymes and their poor absorption. The overall process of oral delivery is frequently impaired by several physiological and pharmaceutical challenges that are associated with the inherent physicochemical nature of the drugs and/or the variability in GI condition such as pH, presence of food, transit times, as well as enzymatic activity in the GI tract.

In order to overcome the above obstacles, the ideal oral delivery system must release its contents in a pH-dependent fashion only at the optimal target region, remain in the optimal site long enough for the complete peptide and protein release to be absorbed across the intestinal epithelium, and have a reproducible therapeutic effect. Thus, site specific delivery is required to deploy the peptides and proteins intactly to specifically targeted parts of the body through a platform that can control their release by means of physiological and/or chemical triggers [2-4].

In recent years, a number of drug delivery companies specializing in protein delivery have evolved. Some of these companies are attempting to attain suitable formulations such as liposomes or microspheres to protect peptide and protein drugs from enzymatic degradation in the GI tract or use site-specific delivery to the colon to

by pass the harsh GI environment. Moreover, study of these problems and challenges is considered an important strategy for improving oral drug delivery that requires thorough understanding of the drug's physicochemical properties, GI physiology and biochemistry, polymer science, pharmacokinetics and pharmacodynamics.

2. Required parameters for effective oral protein delivery

In order to obtain sufficient peptide absorption in the gut, novel delivery systems must be developed that are able to overcome the barriers present in the oral route. The main barriers include: the acidic environment in the stomach, the digestive and proteolytic enzymes in the small intestine, the low permeability of the intestinal epithelium to large hydrophilic peptide molecules and finally the first pass metabolism of the peptides in the liver [5-8].

Protease inhibitors such as Aprotonin, Chymostatin, EDTA and Leupeptin can be used to locally deactivate the proteolytic enzymes of the GI tract. In order to be safe, however, these protease inhibitors have to be linked to high molecular weight hydrophilic matrices to avoid their absorption and possible cell toxicity [9-11].

Peptide and protein protection can also be achieved using chemical modification of the peptides at their $-NH_2$ or -COOH terminus. Lipophilic derivatives of peptides such as monocaproyl and dicaproyl derivatives of insulin as well as glycosylated insulin can enhance the protein permeation across the intestinal membrane and hence protect the protein from deactivation [12].

Moreover, permeation enhancers have been used to reversibly open the tight junctions of the intestinal epithelium and allow the passive absorption of peptides and proteins by the paracellular pathway. Two main classes of materials including calcium chelators and surfactants are able to increase the permeability of tight junctions and thus improve the absorption of macromolecules and hydrophilic substances. While chelating agents may induce disruption of actin filaments by extracellular Ca^{+2} depletion, surfactants cause irreversible exfoliation of the intestinal epithelium. However, neither of these substances can be used as permeation enhancers for hydrophilic macromolecules such as peptides or proteins due to their interactions with phospholipid bilayer of the intestine and the resulting cell toxicity [13].

Mucoadhesive polymers such as chitosan and trimethyl chitosan (TMC) have mucoadhesive properties that enable them to attach to the intestinal membrane and interact with the actin filaments of the tight junction to reversibly open them and allow for the passage of hydrophilic peptides across the membrane. These mucoadhesive polymers are shown to be non toxic and due to their specific interaction with the actin filaments and lack of interference with the phospholipid bilayers of the enterocytes, their use is becoming very common for the induction of the paracellular transport of hydrophilic macromolecules within the opened water filled channels. Their possible use will be discussed in more detail later in this chapter.

Microparticulate and nanoparticulate drug delivery systems have attracted an immense attention as novel carriers for the delivery of lipophilic and hydrophilic substances as well as vaccines [14]. There is a strong belief that nanoparticles of appropriate size may pass the mucosal membranes intactly and deliver their drug load into the systemic circulation. In the case of hydrophilic drugs, nanoparticles should be able to protect such drugs from degradation in the intestinal fluids and improve their penetration and permeation across the intestinal mucosal epithelium [15-17]. Suitable nanoparticles have mucoadhesive properties which are due to their particle size and the particle's surface charge. However, more and more research shows that only a small fraction of nanoparticles is able to act as a carrier for hydrophilic drug molecules across the enterocytes and to deliver their drug load at the serosal site, which in most cases was not sufficient for a therapeutic effect. It was further shown that a substantial part of the nanoparticles may be internalized into the intestinal epithelial cells [18, 19]. Exception is the transport of antigen containing micro and nanoparticles across so-called M-cells with specified particle uptake mechanism capable to induce a sufficient high immunogenic response. More details about the use of micro and nanoparticulate systems will be given below. The following sections will highlight the possible use of mucoadhesive polymers with special emphasis on chitosan and its increasing family of derivatives as well as promising delivery systems for peroral peptide delivery.

3. Chitosan and its derivatives

Chitosan is a polysaccharide composed of two subunits, D-glucosamine and N-acetyl-D-glucosamine, linked together by ß(1-4)glycosidic bonds. Chitosan, a constituent of crustacean shells and being the second most abundant biopolymer after cellulose is derived from chitin by deacetylation. Chitosan attracts a lot of attention in the pharmaceutical research as a polymeric drug carrier. Chitin and chitosan are copolymers; however, chitin has a limited application because of its poor solubility and reactivity. Chitosan is a fully or partially deacetylated chitin derivative and is consequently soluble in acetic acid and other acidic solvents. This polymer has an apparent pka of about 6.5 and is soluble in acidic solutions with pH values lower than 6.5. In recent years, significant progress has been made in identifying substances, which may increase the absorption of drugs through the paracellular pathways at a wider pH range [20]. The intestinal epithelial cells express apical intercellular attachments, known as tight junctions, connecting the enterocytes with each other. They have some regulation mechanism for the paracellular absorption of hydrophilic compounds like glucose when present in higher amounts and allow the passage of macromolecules through their intercellular space after their opening is triggered externally by specified ionic interactions.

Chitosan with its mucoadhesive and non toxic properties can act as a significant absorption enhancer by opening the intercellular tight junctions of the epithelia and promoting the paracellular permeation

of hydrophilic macromolecules [21]. Nevertheless, chitosan has poor solubility at pH values above 6.5; therefore, water soluble chitosan derivatives, which are soluble in both acidic and basic physiological environments, are good candidates for improving the paracellular permeation of peptide and protein drugs in the whole GIT. However, just recently a chitosan product has been synthesized by controlled deacetylation process of chitin which results in a soluble chitosan at pH value 7.2. (Personal communication, Prof. Jörg Thöming, University of Bremen, Germany).

Different studies were carried out to synthesize and determine the antibacterial activities of quaternary ammonium salt of chitosan. These investigations showed that the antibacterial activities of quaternary ammonium salt of chitosan are much stronger than that of chitosan itself since the cationic charge of the ammonium salt is found to increase the interaction with the negative peptidoglycan residues of the bacterial cell surface and will make the bacterial membrane more permeable [22, 23].

Trimethyl chitosan (TMC) was initially synthesized and characterized by Sieval et al [24]. Further in-vitro and in-vivo studies of the intestinal absorption of octreotide by quaternized chitosan showed that TMC is able to increase the permeability of octreotide across the Caco-2 cell monolayer [25]. Moreover, Thanou et al. have studied the effect of TMC on the intestinal permeation of buserelin. This investigation has shown that TMC, as permeation enhancer, similar to chitosan, is able to open tight junction in a reversible way and

increase the permeation of buserelin across the intestinal epithelia both in vitro and in vivo [26]. Moreover, Jonker and coworkers have studied the intestinal paracellular permeation enhancement with TMC of various substitution degrees [27]. Their studies clearly demonstrated that their TMCs were able to enhance the intestinal permeation in a neutral pH environment. Furthermore, it was shown that the degree of quaternization of the derivative has a major impact on its permeation enhancing properties across the intestinal epithelia. Due to their unique properties, such as their permeation enhancing effect and enzyme inhibitory capabilities, chitosan and its derivatives also act as antimicrobial agents [23]. These investigations have shown that growth inhibition of chitosan against microorganisms, such as fungi and bacteria depends on the molecular weight of chitosan [22].

Avadi et al. have synthesized two new derivatives of chitosan, namely triethyl chitosan (TEC) and diethyl methyl chitosan (DEMC) using a factorial design approach to achieve the optimum experimental conditions [28, 29]. They have investigated the effect of TEC on permeation of brilliant blue across intestinal epithelia (ex vivo). Their investigation indicated a significant increase in absorption of sodium fluorescein and brilliant blue in the presence of TEC compared to chitosan. TEC bearing a positive charge is therefore also able to interact with the tight junctions of colon and increase the permeation of the model drug via the paracellular pathway [28]. Moreover, the enhancing effect of DEMC on insulin absorption from the ascending colon in rats was investigated in vivo [30]. These studies have shown

that there was a significant decrease in blood glucose levels after 60 min, when a mixture of insulin and polymer was injected into the ascending colon. They have claimed that chitosan derivatives may induce a redistribution of cytoskeleton of F-actin. Furthermore, it may be possible that the polymers interact with the proteins of the tight junction (e.g. occludin and claudin) leading to disruption of tight junction integrity and a higher permeation of the insulin across the intestinal membrane. Their investigation has shown that DEMC with cationic character is compatible with insulin and no precipitation or aggregation phenomena were observed during the preparation of insulin-DEMC mixture in phosphate buffer solution at pH 7.2 [30, 31].

Bayat et al. have synthesized and fully characterized dimethyl ethyl chitosan (DMEC), another new chitosan derivative with a substitution degree of 50±5% [32]. In their investigations, insulin nanoparticles were prepared using the polyelectrolyte complexation method between insulin and DMEC or chitosan for further ex vivo and in vivo studies in rats [33]. They have shown that insulin in the nanoparticulate form is more permeable across the rat's colon to decrease the blood glucose level mostly due to the endocytotic pathways whereby the insulin containing nanoparticles are transported across the colonic membrane resulting in an approximately 2- fold reduction in blood glucose level compared to the free insulin [34]. Trimethylated and triethylated 6-NH_2-6-deoxy chitosan with substitution degrees of 50±5% were synthesized and

characterized by our group [35]. These derivatives contain 2 alkyl groups at their C_2 and C_6 positions and are water soluble. The permeation enhancing effect as well as the cytoxicity of the C_2-C_6 methylated 6-NH_2-6-deoxy chitosan was compared with those previously studied with TMC. It was shown that the new chitosan derivative, trimethylated 6-amino-6-deoxy chitosan, at 0.5% concentration, reduced cell numbers in water soluble tetrazolium (WST-1) cytotoxicity assay compared to the control, indicating a certain enhanced cytotoxicity as compared to TMC. Moreover, despite its higher zeta potential due to the presence of two positively charged methyl groups per monomer units, trimethylated 6-amino-6-deoxy chitosan did not significantly reduce the TEER in confluent monolayers as compared to the control. This could be explained by the higher susceptibility of Caco-2 cells in a 24 hrs culture to the cytotoxic polymer properties compared to fully differentiated epithelial cells forming tight monolayers. Moreover, the bulky size of the polymer with reduced flexibility of its backbone and the possibility of the two positive groups' repulsion may cause a less significant reduction in TEER (unpublished results).

The bioavailability, biodegradability and the extensive studies on chitosan and its numerous derivatives have made them, as multifunctional polymeric permeation enhancers, good candidate polymers for oral peptide/protein delivery.

4. Novel Drug Delivery systems

Today, the design and development of novel peroral delivery systems for peptides and proteins are the main goal of many pharmaceutical researchers. The low oral bioavailability of these peptide and proteins due to their low permeation across the intestinal epithelium, the harsh environment of the gastric pH, their rapid degradation by the proteolytic enzymes and their rapid clearance due to the first pass effect are the major drawbacks of developing a successful delivery system, however the delivery of the hydrophilic peptide drug is not difficult to achieve but to enable its absorption in the intestinal tract is the crucial part. Hence, the delivery system has not only to overcome the harsh pH of the stomach and the enzymatic degradation of the proteins in the GI tract, but to also increase the permeation of these molecules across the GI epithelium either by opening the tight junctions and increasing the paracellular transport or by increasing the endocytotic passage of the molecules through intracellular transport. In order to achieve this, the delivery system must be able to attach to a specific site in the GI tract long enough for the drug to permeate across the epithelium before the delivery system is being detached by the peristaltic movements of the gut [36].

A number of peroral delivery systems were designed using liposomes, beads, adhesive drug delivery systems, superporous hydrogels, chitosan and its derivatives as well as nanoparticles to protect the

drugs from the harsh environment of the GI tract and prolonging the drug's transit time at a specific site of the GI tract for an optimum drug bioavailability.

4.1. Liposomes

Long circulating macromolecular carriers such as liposomes can exploit the enhanced permeability and retention effect for the protein drugs. Liposomes are vesicles consisting of one to several, chemically active lipid bilayers. Drug molecules can be encapsulated and solubilized within these bilayers. Different types of phospholipids such as phosphotidyl choline or phosphotidyl inositol may be used in liposomal carriers. Liposomes are prepared by sonication, reverse phase evaporation or film formation [37, 38]. Among different types of liposomes, dehydrated-rehydrated vesicles are most commonly used in protein drug delivery due to the ease of preparation and low amount of stress applied to the proteins [39]. The liposomes can be easily decorated with targeting moieties, e.g., antibodies, hence delivering the protein drugs to their specific target site [40, 41]. The liposomal composition, encapsulation efficiency, the rate of drug release from lipid bilayers, size and the surface charge are all important factors in successful liposomal drug delivery [38]. Stefanov et al. have used liposomes prepared from phosphotidylcholine (PC) and cholesterol (CH) for oral insulin delivery. They have reported a significant reduction in blood glucose levels in diabetic rats. Further investigations with liposomes containing insulin in rats and dogs showed reduction in blood glucose levels [43, 44]. Although

liposomes with their organized structures have some advantages as drug delivery systems, the extensive leakage of water-soluble drugs entrapped in liposomes during the GIT passage, the low drug entrapment, the heterogeneity of the vesicle size, the poor reproducibility and instability of formulations are some of the disadvantages of using liposome as peptide/protein drug delivery system.

4.2. Microtablets

Microtablets with diameters of 0.5-3mm containing permeation enhancers and/or enzyme inhibitors were designed and investigated for the peroral delivery of protein and peptide drugs. The permeation enhancers must be released rapidly from the dosage form and prior to the release of the peptide over a wide area across the epithelium. In order for the peptide to pass through the epithelium, the site of opening of the paracellular pathway must coincide with the site where the peptide is released from the dosage form [45]. Hence, multiple unit dosage forms (MUDFs) were designed to control the release of the drug [46, 47]. The minitablets can be then filled in gelatin capsules and enteric coated to be protected from the acidic condition of the stomach. Microtablets are easy to manufacture, can be used in defined sizes and strengths and show low variability within a batch [48]. In a study by Van der Merve et al. minitablets containing TMC permeation enhancer and Desmopressin (1-(3-mercaptopropionic acid)-8-D-arginine Vasopressin monoacetate (DDAVP) were designed. The release of both DDAVP and TMC from different

formulations of multi tablets was investigated [45]. The results suggested that suitable DDAVP release was obtained only from formulations that were too big to be fit inside the largest available gelatin capsule rendering them unsuitable for in vivo usage.

4.3. Microspheres

Spherical microspheres, prepared by complexation between oppositely charged macromolecules such as chitosan and negatively charged molecules such as tripolyphosphate (TPP) or alginates have received a lot of attention as drug delivery vehicles for protein drug delivery purposes [49, 50]. These microspheres can protect the drugs from the hostile environment of the GI tract, improve drug absorption via the paracellular route and control the drug release at a specific site [51-53]. Lueßen et al. and Kotzé et al. have applied drug containing chitosan microspheres on Caco-2 cell monolayers and showed a strong increase in the transport of buserelin, insulin and vasopressin derivative [20, 54]. A number of investigations were done by Shu et al. and Mutara et al. for controlled release drug delivery [55, 56]. They showed that variables such as drug concentration, type and concentration of chitosan, the pH of TPP solution, volume of the internal and external phases, gelation time as well as drying conditions can all determine the fate of drug release from chitosan beads. Avadi et al. have used enteric coated capsules containing Brilliant Blue chitosan beads as model hydrophilic drug for colon drug delivery [57]. The γ-scintigraphy images have demonstrated that Eudragit S coated capsules containing Brilliant Blue loaded–chitosan

beads are suitable for colon drug delivery. It can be thus concluded that the non toxic chitosan microspheres and beads can increase the bioavailability of the peptide and protein drugs by protecting them from degradation, when they are able to mucoadhesively attach to a specific site on the intestinal tract and to increase drug permeation by opening the tight junctions via the paracellular pathway.

4.4. Mucoadhesive Drug Delivery Systems

Mucoadhesion is the attachment of any type of polymer to the mucus layer via strong interaction between the functional groups of the polymer and those of the mucosa lining of the tissue. The mucoadhesive bonding is attained mostly by physical, chemical and more importantly through H-bonding. Hence, the presence of hydroxyl, carboxyl and H-bond forming functional groups strongly contributes to the strength of mucoadhesion [58, 59]. The formation process of mucoadhesive bonds include 1) wetting and swelling of the polymers, 2) interpenetration of the mucoadhesion polymer chains and entanglement of the polymer and mucin chains, 3) interfacial interaction of functional groups, 4) formation of weak chemical bonds [60].

The use of mucoadhesive drug delivery systems results in a controlled drug release and attachment at a specific site of the body. Increasing the residence time of the drug delivery systems at the site of absorption in the body may result in prolonging their action. As the GI tract is covered by a mucus layer, the mucoadhesive drug delivery system must be able to attach to a specific site in order to be

beneficial. Acrylic acid based polymers have been used extensively for mucoadhesive applications. Their strong bond strength in contact with tissues allows localization of the drug at the site of absorption, increasing residence time at the absorbing tissue and increasing drug bioavailability. Their responsive behaviour to different pH values allows the drug to be released at the desired site of the GI tract [58, 61, 62].

In order to increase mucus interpenetration, adhesion promoters such as polyethylene glycol (PEG) may be employed, which are not mucoadhesive but contribute to the adhesion process. Moreover, these tethered promoters may be grafted onto polymeric surfaces such that at the one end they are covalently attached to the polymer surface and the other end is free. These grafted chains are able to diffuse into the mucus layer and enhance the mucoadhesiveness of the system [63, 64].

Peppas et al. have done extensive studies on the design and the effect of network morphology of polyethylene glycol (PEG) tethered copolymers as novel mucoadhesive drug delivery systems [60]. They have suggested that the performance of the copolymer is due to the synergistic effect of both polymers: the backbone polymer providing the hydrogen bonds between the hydrogel and mucus layer as well as the adhesive promoter that contributes to the mucoadhesion by increasing the chain interpenetration.

Furthermore, oral insulin delivery was investigated using hydrogels of poly(methacrylic acid-g-ethylene-glycol) P(MAA-g-EG) by Peppas et al. [65]. A hypoglycemic effect combined with insulin absorption was observed in rats. These results could be due to the ability of the complexes to entrap and protect the drugs in their network structure. At the pH present in the small intestine, these complexes dissociate and the network swells and releases the peptide [66-68]. Moreover, these hydrogels were shown to decrease the TEER (transepithelial electrical resistance) across the Caco-2 cell models with no sign of cytotoxicity [68]. The in vivo studies were done in male Sprague-Dawley rats using a closed loop absorption method. 25IU/kg of human recombinant insulin was incorporated into the polymer and infused in an isolated illeal segment. The control sample contained the polymer without insulin. At predetermined intervals blood samples were withdrawn from the jugular vein, serum was collected and the insulin levels were determined by enzyme immunoassay. The insulin bioavailability of the formulation was measured using subcutaneous injections. The bioavailability of insulin was shown to increase to 6.2% compared to the control [65]. The mucoadhesion of their delivery system is mediated by weak, non covalent bonds such as hydrogen bonds, van der Waal's forces and ionic interactions resulting in mucoadhesion that may not be strong enough to localize the hydrogels at the specific site for a sufficiently long time. This suggests that although p(MAA-g-EG) hydrogels are promising carriers for oral insulin delivery the bioavailability of the protein is still too low for the system to be commercialized.

Thiolated polymers are another promising class of mucoadhesive with their capability to form strong covalent bonds through the disulfide binding of the polymers with the mucus gel layer of the mucosa. Thiomers are mucoadhesive polymers with thiol bearing side chains [69]. The disulfide bonds are formed between both the cysteine side of the polymers and glycoproteins of the mucus layer as well as the thiomer itself leading to a strong adherence to the mucus gel layer [70]. At physiological pH values, the oxidation of the thiol groups results in gelling properties due to the formation of inter- and intramolecular disulfide bond. Studies on thiolated poly (acrylic acid) in comparison to the unmodified polymer have shown that the mucoadhesive properties of the polymer measured using tensile studies and by using rotating cylinder method were 20 fold increased. Furthermore, the residence time of the polymer in the small intestine was prolonged by up to 3-fold through immobilization via the thiol groups [71, 72]. Thiolated chitosan was shown to improve mucoadhesion by more than 100- fold [73, 74]. Recently, dosage forms based on thiomers using peptide microparticles were generated via solvent evaporation emulsification method. Because of the formation of the disulfide bonds within the particles they did not disintegrate under physiological conditions for 48hrs and the mucoadhesive properties of the microparticles were improved 3- fold due to immobilization of the thiol groups compared to the control group consisting of the peptide alone [75]. Kast et al. have used thiolated polycarbophil for oral delivery of low molecular weight heparin (LMWH) in rats. In their investigation, they have shown that

the absorption of LMWH was significantly increased using the thiolated polymer in comparison to the unmodified polymer. They have reported a 19.9±9.3% bioavailability in rats compared to the intravenous application [76].

Marschütz et al. have investigated the oral insulin delivery using thiolated polycarbophil in diabetic mice. The results indicated that a significant decrease was observed in blood glucose level when insulin was administered using the thiolated polymer in comparison to the unmodified polymer [77]. Similar results were obtained when pegylated insulin was incorporated in thiolated polycarbophil and orally administered to mice. The results indicated that the decrease in blood glucose level was maintained up to 24 hrs and the oral pharmacological efficacy of this mucoadhesive system compared to the s.c. injection was 7% [78]. Accordingly, while thiomers seem to be suitable for oral peptide delivery, their low stability in aqueous form as well as their biosafety issues are amongst the disadvantages of these systems as oral delivery vehicles. Furthermore the rapid mucus turnover may be responsible for a reduced residence time at the site of absorption despite the formation of covalent –S-S- bonds.

4.5. Nanoparticles

Today, a vast number of investigations have been focused on nanoparticles and their role as drug delivery vehicles. Nanoparticles were first introduced in the mid seventies by Birrenbach and Speiser [79]. The preparation of nanoparticles was simple, the particles

formed were relatively stable and easily freeze-dried; hence, biodegradable polymers were found useful and further developed for drug delivery. Polymeric nanoparticles have the advantages of protecting the protein and peptide drugs from chemical and enzymatic degradation in the GIT, increasing their stability and absorption across the intestinal epithelium as well as controlling the drug release [80-83]. A number of techniques such as polymerization, nanoprecipitation, inverse microemulsion can be used to prepare polymeric nanoparticles; however, most of these methods involve the use of organic solvents, heat and vigorous agitation which may be harmful to the peptide and protein drugs [84, 85]. More recently the ionic gelation technique is used as the most favorable method for producing peptide and protein nanoparticles. The nanoparticles prepared by this method have a suitable size and surface charge, spherical morphology as well as a low polydispersity index indicative of a homogenous size distribution. The lack of using organic solvents, sonication or harsh conditions during preparation reduces the damage to the peptide and proteins and makes this method a favorable one for the preparation of protein loaded nanoparticles [86, 87]. Chitosan nanoparticles with excellent biodegradable and biocompatible characteristics have been used extensively as drug delivery vehicles [88]. However, due to poor solubility of chitosan at pH above 6.0, its quaternized derivatives such as trimethyl chitosan, triethyl chitosan, diethylmethyl chitosan and dimethylethyl chitosan, which are soluble at the intestinal pH, have been used to prepare nanoparticles loaded with insulin [24, 28, 29, 32]. Chitosan nanoparticles loaded with

insulin were prepared by mixing the positively charged polymer with the negatively charged insulin and nanoparticle formation occurred via electrostatic interaction. Studies have shown that insulin in nanoparticulate form was more likely to be delivered across the GI tract than in its free soluble form [33, 34, 89]. The particle size and surface charge are critical factors in nanoparticle absorption. Size is a determining factor for both uptake and biological fate of the particulate systems. Moreover, a size dependent phenomenon exists in the gastrointestinal absorption of the particles. Studies have shown that particles with a size of 100nm were taken up 6- times more than particles with 100µm by the absorptive cells [90-92]. Hydrophobic particles are absorbed more readily than hydrophilic ones. Thus increasing the hydrophobicity of particles may enhance their permeability through mucus but decreases the translocation through and across the absorptive cells [93]. In the GIT, the particles interact with the mucus before coming into contact with the absorptive cells. Positively charged particles are more prone to uptake as they can associate with the negatively charged functional groups in the mucus [94]. Accordingly, biodegradable, hydrophobic nanoparticles with sizes between 100-200 nm and positive surface charge may be good candidates for uptake by the epithelial cells. The disadvantage of the nanoparticles is their bigger size in comparison to the soluble protein molecules and the fact that a lot of particles may get trapped inside the cells within the cellular membrane such as the Golgi apparatus or the endoplasmic reticulum being unable to pass across the cells intactly. Accordingly, while the use of nanoparticles is highly

recommended for gene therapy their use for peptide drug delivery and absorption is debatable also with respect to the minimal drug load they can carry with them in comparison to bigger particles.

Even though all of the above mentioned delivery systems gave reasonable results when studied in-vitro and ex-vivo, they were only tested in-vivo using small animals such as mice and rats with intestinal diameters much less than that of humans. For a delivery system to be useful for commercialization, it must have reasonable bioavailabilities in bigger animals such as pigs or dogs with intestinal diameters closer to that of the humans. The small intestinal diameter of smaller animals allows the delivery system to easier come in direct contact with the intestinal wall where in bigger animals and humans reaching the absorbing surface still with full mucoadhesiveness is a big challenge. Moreover, the amount of mucus produced in bigger animals is higher than in the GIT of smaller animals. It was shown for nanoparticles developed by Peppas et al. that they lose their mucoadhesive properties in contact with soluble mucins present in the GIT of larger animals before reaching the absorbing surface and are no longer able to open the tight junctions and allow for the paracellular transport of the drugs [95]. Hence, to overcome the above obstacles, delivery systems using superporous hydrogels and gas empowered delivery systems were designed and examined in larger animals such as pigs, rabbits and humans.

4.6. Super porous hydrogels (SPH) based delivery systems

SPH and SPH composites were synthesized by Park et al and adopted by Dorkoosh et al. for intestinal drug delivery [96, 97]. These polymers are able to swell very rapidly up to 200-fold of the original volume upon sucking up the gut fluids and are then able to attach mechanically to the gut wall and bring the dosage form in close proximity of the site of absorption. Consequently, these polymers not only increase the residence time of the dosage form at a specific site in the gut; but also by absorbing the gut fluids they decrease the enzymatic activity. The SPH and SPHC delivery systems were prepared either by inserting the core inside the conveyor system (i.e. core inside, c.i.) or attaching to the surface (i.e. core outside, c.o.). The core consists of the peptide/protein drug, such as octreotide or insulin. These formulations were then placed in gelatin capsules and enterically coated with Eudragit S100. Hence, the delivery system could safely pass through the acidic environment of the stomach and thereafter dissolved in the *responsive* pH of the small intestine allowing for the polymer to swell and mechanically attached to the gut wall. The mechanical pressure on the intestinal cells opens the tight junctions allowing for the paracellular transport of the peptides across the intestinal membrane [98]. In vivo studies in pigs of a weight of about 25 kg have shown that using this delivery system, the bioavailability of octreotide was increased up to 16% in comparison with the peroral administration of octreotide without polymer [99]. However, the synthesis and fabrication of the delivery systems is based on SPHs or SPHCs technology, which is difficult and currently

not commercially feasible on mass production scale. Moreover, their big size (i.e. capsule size 000), is not easily swallowed and may cause variable residence times in the stomach of the patient.

4.7. Gas Empowered Drug Delivery system (GEDD)

The latest approach for a novel drug delivery device is using Gas Empowered Drug Delivery (GEDD) system to deliver hydrophilic drugs such as peptide and proteins to the intestinal tract and enhance their absorption across the intestinal wall by pushing the active compounds together with the mucoadhesive polymer polyethylene oxide (PEO) and TMC as penetration enhancer to the absorbing membrane of the gut tissue using CO_2 gas. The drug will be then adhered to the mucus layer together with the PEO to prolong the residence time at the mucosal surface and TMC as permeation enhancer will simultaneously trigger the opening of the tight junctions for enhancing drug permeation by the paracellular pathway. The mucoadhesive remnants of the delivery system slide down the mucus membrane and will be shed off at the latest in the large intestine where the dosage form is degraded and expelled. In the GEDD system CO_2 acts mainly as the driving force to push the delivery system to the absorbing membrane. Additionally, it can form a layer around the delivery system protecting it from enzymatic and proteolytic degradation. Furthermore, the CO_2 bubbles may act as a permeation enhancer that mechanically opens the tight junctions [100]. In order to protect the drug from the acidic pH of the stomach, the GEDD system was enterically coated with cellulose acetate

phthalate (CAP). An increase in insulin permeation using this delivery system may be due to the synergistic effect of both the CO_2 and TMC in the form of mechanical and chemical enhancement, respectively. The advantage of this delivery system over the superporous hydrogel is its ease of production in large scale.

5. Conclusion

Numerous investigations were done to develop suitable peroral delivery systems; nevertheless, to date no single delivery system has been designed to cover all the criteria required for peptide drug delivery. Developing a suitable peroral drug delivery system requires a multi task planning. The delivery system must first and foremost overcome the harsh acidic environment of the stomach. Secondly, it must protect the peptide and protein drugs from the enzymatic degradation in the intestine. Once in the intestinal tract, it must be able to adhere to a specific site long enough to be effective. As the delivery system attaches to the intestinal epithelium it must act as an enhancer to open the tight junctions and enhance the drug permeation across the intestinal wall via the paracellular pathway. Multifunctional polymers such as polyacrylates and chitosans with their multiple derivatives show promising properties as penetration enhancers for the paracellular absorption route of hydrophilic macromolecules by reversibly open the tight junctions; however, their high viscosity and slow dissolution rates make the development of suitable delivery systems using these polymers very difficult. The bioavailability of peptide and proteins crossing the intestinal wall

must be enough to have a therapeutic effect. Moreover, the delivery system must be easy to prepare and to scale up in order to be appropriate for commercialization. In short, the concept of a suitable peroral peptide delivery system is still far from reality and requires a lot of investigations to become reality.

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